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COMMENTARY

Pancreatic Islet Transplantation: An Update

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Clinical islet transplantation has recently received a strong impulse from the results obtained with the introduction of a glucocorticoid-free immunosuppressive regimen leading to insulin independence at 1 year in 100% of the treated patients. Thus, islet transplantation may now be considered as a viable routine option for the treatment of insulin-dependent diabetes mellitus. As an alternative approach to endocrine cell replacement, it displays several potential advantages over whole-organ transplant. Indeed, islet transplantation is at present a minimally invasive procedure and offers for the future the unique possibility of being performed under donor-specific tolerant conditions because islets may potentially be engineered in vitro. In addition, various approaches such as in vitro islet expansion, the use of immortal beta cells, engineered surrogates, or xenogeneic islets could make the availability of donor tissues unlimited. However, most of these approaches are still to be realized in the clinical practice and several problems with the current procedure still need to be addressed to optimize certain aspects, such as organ procurement and preservation, islet isolation and culture, modality of transplant, and immunosuppression. Indeed, pancreata from multiple donors are still needed to guarantee a sufficient islet mass because a substantial number of transplanted islets fails to engraft into the liver. Overcoming these obstacles may dramatically expand the number of transplants and even minor changes applied to the steps of the procedure may produce significant impact in the short run. In light of the renewed enthusiasm in the field, the focus of the present update is to review the articles published in *Cell Transplantation* since 1999 and to analyze the scientific contribution the journal has made to the field as this new era for islet transplantation begins.

The primary protocol of islet isolation was established more than a decade ago with the introduction of the automated method. This method consists in loading the pancreas into a digestion chamber filled with collagenase solution, and allowing for progressive islet release in a continuous digestion process. From that point on, several modifications were adopted in order to improve islet recovery. The delivery of collagenase within the pancreas is performed by intraductal perfusion of the enzyme, allowing distension of the parenchymal tissue. Appropriate diffusion of the collagenase solution from the ductal tree into the peri-islet interstitial matrix in a different portion of the gland depends on several factors, including perfusion pressure, temperature, time, and extracellular matrix composition. Several of these parameters are difficult to control or determine. This is the reason why Lakey et al. recently introduced a recirculating device system (10) that provides control over perfusion pressures and collagenase temperature. With this procedure the enzyme solution is maintained cold (4°C) throughout the perfusion time and injection pressure is monitored and properly adjusted. The obtained results were proved to be superior in terms of human islet recovery compared with standard retrograde injection using a syringe.

Once isolated from the pancreas, islets can either be immediately transplanted or cultured for a defined period of time before the transplant. This latter approach may provide several potential advantages over immediate transplant, such as the possibility to analyze the islets' functional capabilities, to further purify them from

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the exocrine contamination, or to manipulate them with gene therapy tools. For these purposes, ideal culture conditions after isolation should at least replace the loss of critical factors required for islet function and survival, most of which are still unknown. Several lines of research involving different approaches stemmed from this notion. One approach is aimed at restoring the natural extracellular interactions between islets and their microenvironment. It is well established that integrins, the surface receptors for extracellular matrix components, are able to transduce essential signals for cell function and survival. Indeed, several cell type-specific functions may be impaired in the absence of integrin–matrix interaction, because the intracellular domains of these receptors specifically interact with cytoskeleton components and influence its distribution within the cell. Furthermore, lack of integrin engagement may result in a specific apoptotic process, named "anoikosis," which affects tissue cells deprived of surface contact. In this view, Nagata et al. attempted to reconstruct cell–collagen interaction in isolated rat islets (12). Indeed, they were able to show that glucose-induced insulin secretion of islets cultured with collagen type I gel or a mixture of collagen type I and IV was improved after 11 days in culture. These results further underline the importance of artificially preserving integrin-mediated signaling during islet culture as a tool for improving beta-cell function and survival.

Recently, an improved in vitro culture media composition has been introduced for pretransplant preservation of human islets, allowing successful transplantation in patients with type I diabetes (1). In fact, there are now several patients at our institution and elsewhere who have become insulin independent following transplantation of human islets cultured for 1–3 days on these conditions before intraportal infusion.

Because the islet isolation procedure may induce relevant changes in islet behavior and viability, leading to direct consequences on the transplant outcome, several investigators have focused on setting rapid, accurate, and objective measurements of islet viability after isolation and culture. Indeed, Miyamoto et al. examined the features of fluorescein diacetate/ethidium bromide staining in viable cells and established a direct correlation between islets and their insulin content (11). In a different functional approach, Kanazawa et al. measured adenylylcyclase rat islet cell activity (7), because cAMP plays an essential role in determining islet beta-cell viability and responsiveness to various hormonal stimuli. The correlation of the results with posttransplant islet cell function suggested that this assay is a valuable tool to assess viability and perhaps efficiently predict islet transplant outcome.

So far, transplanted islet tissue has been derived, almost without exception, from cadaver organ donors. Despite any attempt to maximize the donors/recipients ratio in islet transplantation, the limited supply of organs will always represent a major obstacle for the diffusion of this therapeutic approach for diabetes. This notion boosted the efforts to identify potential new sources of insulin-producing cells that may act as surrogates for natural beta cells. A promising line of research stemmed from the observation that human pancreatic ductal cells may differentiate in vitro into insulin-producing cells. In this respect, isolation and culture conditions are critical to generating an abundant ductal cell supply, as shown by Gmyr et al. (6). Another strategy is aimed at genetically engineering cell lines with pro-insulin cDNA and testing their ability to substitute for islet function. Indeed, pituitary GH3 cell clones transfected with human pro-insulin restored normoglycemia when transplanted in streptozotocin-treated mice (4). Concerns still remain as to the safety of transplanting immortalized/transformed cell lines and on the ability of non-beta cells to control insulin secretion.

Current clinical islet transplantation protocols have selected the liver as the preferred site of islet implantation by direct delivery through the portal vein. Although universally adopted, this option still has several pitfalls. Indeed, portal vein thrombosis still represents a serious complication during the islet transplant procedure. Moreover, several lines of evidence suggest that most transplanted islets are rapidly destroyed by an early nonspecific cell-mediated inflammatory injury response. In fact, islets are forced to graft in an environment that is extremely reactive due to the abundancy of Kupffer cells. This cell type possesses both proinflammatory activities and antigen-presenting capabilities. The interaction of islets and acinar tissue with Kupffer cells has been explored by Clayton et al. in an in vitro coculture model (2). In this study, secretion of proinflammatory mediators such as thromboxane B_2, prostaglandin E_2, 6-keto-prostaglandin $F_{1\alpha}$, and prostaglandin $F_{2\alpha}$ by Kupffer cells was observed after coincubation with islets and acinar tissue. These mediators participate in the inflammatory cascade that leads to the production of harmful cytokines, such as TNF-α, and oxygen free radicals. Preventing the activation of this phenomenon is therefore of paramount importance in reducing the impact of primary nonfunction. Because the use of common anti-inflammatory agents, such as steroids, may be detrimental to islet function, the selective targeting of critical molecules may represent an invaluable approach to counteract the inflammatory process. Blockade of TNF-α is of particular relevance due to its dual properties of inducing apoptosis of target cells and activating the inflammatory cells. Indeed, infection of human islets with an adenovirus construct encoding an inhibitor of TNF-α

was effective in limiting damage to beta cells induced by leukocytes in vivo (5). Notably, this ability to manipulate islets in culture before transplant allows investigators to exert a specific local inhibition of this cytokine, thus avoiding the drawbacks of a systemic therapy. Leukocyte infiltration may also be prevented by blockade of critical adhesion molecules that mediate their extravasation into tissues. Because the mechanisms of leukocyte recruitment are also implicated in cell-mediated rejection (3) and autoimmune response, interfering with adhesion molecule function may also be beneficial for these pathologic conditions. One of the most suitable targets is ICAM-1, a cell surface adhesion structure expressed mostly on endothelial cells. Its role is to assure firm adherence of activated leukocytes to endothelium after rolling. Indeed, improvement in islet allograft survival and function was obtained by its targeting (8). Notably, this effect was obtained using either monoclonal antibodies or antisense oligodeoxynucleotides. However, the choice of plasmid or adenoviral vectors for cDNA transfection often proved to be unsatisfactory for their potential cytopathic effect or their inability to adequately transfect nondividing cells such as beta cells. Recently developed lentivirus-based vectors that infect and replicate in nondividing cells could be more suitable for gene transduction into islets. In addition, vectors based on nonpathogenic strains of coxsackiae virus, which are well known to produce sustained infection of islet cells, may provide further tools to engineer beta cells before transplant.

Once engrafted, intrahepatic islets are neovascularized by a mix of hepatic artery and portal vein blood containing presumably low amounts of oxygen, a condition that may negatively influence their function. Lack of neovascularization has always undermined the option of grafting islets in the subcutaneous tissue, a site that would exhibit substantial advantages in terms of simplicity and low morbidity. A successful approach to overcome this problem was explored by Kawakami et al. (9). By delivering bFGF, a potent inducer of angiogenesis, in the subcutaneous site of the graft through a meshed releasing device, they observed the development of a thick, well-vascularized capsule and maintained normoglycemia for more than 1 month in streptozotocin-induced diabetic Lewis rats. Control rats failed to achieve normoglycemia.

In summary, the studies published in *Cell Transplantation* in the past 3 years are important contributions that have paralleled the recent renewed interest in islet transplantation. The potential short-term impact of technical innovations and advances in molecular and cell biology will further contribute to the advancement of the field.

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